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FOLIAR ANATOMY OF THE FAMILY ACANTHACEAE
I. THE TRIBE JUSTICEAE

SHASHI KUMAR* AND G. S. PALIWAL

Department of Botany, University of Delhi, Delhi

ABSTRACT

The venation type, areole size, vein endings, vein tips and epidermal character have been described in 11 species of the tribe Justiceae of the family Acanthaceae. The features like unilacunar node, a prominent strand, venation patterns, structure of the epidermis, diamesogenous stomata, trichomes, lithocysts, etc. have been found to be useful in taxonomic considerations.

INTRODUCTION

The family Acanthaceae comprises about 240 genera and 2,000 species (Willis, 1966). Its members are restricted mostly to subtropical forests, especially inhabiting damp and marshy places. Some genera like *Acanthus*, *Barleria* and *Blepharis* are xerophytic.

Bremekamp (1965), using floral morphology, palynology, and other related characters, has attempted delimitation and subdivision of Acanthaceae. The Justiceae has been subjected to detailed cytological and anatomical studies by several investigators among others including Pant & Mehra (1963), Paliwal (1966), Datta & Maiti (1969), Inamdar (1970), and Ahmad (1974).

The present study, which deals with the foliar anatomy of 11 species, has been undertaken to supplement our present knowledge of the taxonomy of the tribe Justiceae.

MATERIALS AND METHODS

The following species were collected from Old Delhi Ridge and Delhi University Campus:

Subtribe Andrographideae	1. *Andrographis echioides* Linn.
„ Barlerieae	1. *Barleria prionitis* Linn.
	2. *Crossandra infundibuliformis* Linn.
Asystasieae	1. *Eranthemum nervosum* R. Br.
Eujusticieae	1. *Adhatoda vasica* Nees
	2. *Justicia betonica* Linn.
	3. *J. gendarussa* Burm. f.
	4. *J. simplex* D. Don
	5. *Peristrophe bicalyculata* Retz.

Fresh leaves were fixed in FAA and transferred to 5% aqueous solution of NaOH at 37-40°C. The leaves were later rinsed thoroughly with distilled water to remove alkali and brought in a mixture of saturated aqueous solution of chloral hydrate and hydrogen peroxide (1 : 1). Transparent leaves were brought to water through a graded series of chloral hydrate solution. They were washed thoroughly with distilled water and dehydrated in an usual manner by passing through alcoholic series. Staining was carried out in safranin dissolved in a mixture of absolute alcohol and xylene. The preparations were mounted in canada balsam.

For the study of nodal organization, free hand serial sections were taken.

The epidermal preparations were made by peeling or by chemical maceration of leaves in Schulze's fluid. They were mounted in pure glycerine after being stained with haematoxylin.

TERMINOLOGY

In the present work broad categories have been formulated on the basis of number of strands entering the base of the petiole. The term primary, secondary and tertiary veins

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*Present address: Institute of Home Economics, NDSE, New Delhi-110049

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have been given to indicate vein relationships and relative sizes but without any direct morphological implication. In a recent article Dilcher (1974) has referred them as veins of 1°, 2° 3° and so on. Veins departing from the midrib that are smaller than the secondary veins, but stronger than the tertiary have been described as intermediates. The small veins usually form networks which vary in size and shape and subdivide the area of the mesophyll. The smallest areas or regions bounded by the thinner branches of the veins are called areoles and they usually contain blind vein endings. The degree of branching of these vein endings varies markedly in the leaves of different species.

In some leaves dilated tracheidal elements have also been seen at various vein endings in the areoles, more so at the apices. The tracheids which terminate the ultimate tips show an increase in cell diameter and are extraordinarily variable in shape. The tracheidal elements sometimes lying free in areoles have been described as 'free vein endings'.

OBSERVATIONS

All the forms studied of the tribe Justiceae show opposite decussate phyllotaxy, entire margin and unilacunar node (*see* Table 1; Figs. 1 B, I; 3 B).

*Andrographis echioides**, a representative of the subtribe Andrographideae has a single strand at the leaf base and it forms the midrib in the lamina. It gives rise to 6 traces (Fig. 1 A). The two lateral traces on the left cross each other in the beginning and one of these runs parallel to the margin giving out branches which form the plexus at the margin. Almost identical course is followed by the other two traces of the right side, the rest supply the middle region of the lamina. The midrib gives rise to the strands alternately. The secondaries arise at an angle of 45° and branch repeatedly. Thus secondaries, tertiaries, and quarternaries form the vascular network of the lamina (Fig. 1 C).

The areoles are larger at the basal region of the leaf than at the apex (Table 1). The vein endings are of different shapes and sizes because of varying nature of the tracheids composing the vein endings. Some of the side tracheids run throughout the length while others show bendings and a few end in the middle. There is a direct correlation of the vein ending number with the size of the areoles.

The epidermis shows feebly undulated cell walls. In mature stomata the guard cells are arranged at right angles to the subsidiary cells (diamesogenous type; Fig. 1 L and Table II). The trichomes are of two types: unicellular, elongated with pointed tips and round base and those with glandular head and multi-cellular and uniseriate stalk. The lithocysts are elongated with blunt ends (Table II; Fig. 1 D, E).

For the subtribe Barleriae, two representatives-*Barleria prionitis* and *Crossandra infundibuliformis* have been studied. Leaves are caducous and petiolate.

The petiole is triveined in *B. prionitis.* Two fine strands run parallel from the petiole to the middle strand, enter the lamina and ultimately join the midrib at the apex. They give rise to secondaries, tertiaries, and finally quarternaries which ultimately form the areoles and vein endings. The midrib gives rise to secondaries alternately, in the left and right lobes of the lamina. Finally they also follow the same course.

The areole size is variable. They are large at the basal and apical regions and small in the middle. The vein endings per areole are more numerous in the basal part of the lamina (*see* Table I). The vein endings are of different shapes (Fig. 1 H, G) and may comprise one or more tracheidal elements. In some vein endings more than one tracheidal element could also be noticed in different orientations. Variously organized free vein endings also occur.

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*A representative selected for clarity in description

The epidermal cells possess straight to arched walls. Mature stomata are of dia-mesogenous type (for stomatal frequencies and stomatal indices *see* Table II). The trichomes are distributed throughout the lamina and its margin. The interesting feature is of the attachment of the trichomes; their base being fixed on the secondary, tertiary and quarternary veins. They are unicellular with tapering tips (Fig. 1 F). The lithocysts are unicelled to bicelled, with blunt to tapering tips (Fig. H).

The leaf of *Crossandra infundibuliformis* has a single strand at the base of the leaf (Fig. 1 I) and forms the midrib. It branches profusely on either side. The secondaries arise at an angle of 75° and branch again. Thus secondaries, tertiaries and quarternaries form a network coupled with several plexus at the margin. The secondaries form compartments which are quite distinct in the apical portion of the lamina (Fig. 1 J).

As is clear from Table I, the areoles are quite variable in size being smallest in the middle. The vein endings may sometimes become conical or blunt but usually maintain the same thickness throughout (Fig. 1 J, K).

The epidermal cells show arched to sinuous walls (Fig. 1 L). The stomata are diacytic; a few abnormal stomata showed just a single guard cell. The trichomes occur all over the leaf surface but are more crowded at the margins. These are multicellular, usually 2-5-celled and uniseriate with tapering tips. Lithocysts are scanty.

Subtribe Asystasieae which is represented by *Eranthemum nervosum* shows three strands entering the petiole. At the junction of the petiole and lamina, the branches are diverted towards the side of the leaf and the remaining one continues up to the apex as a midrib. The secondaries are given out nearly at 90° of the midrib while most of the tertiaries and quarternaries arise at right angles to the secondaries. At margins, the tracheids form the plexus (Fig. 1 M). The branching on both the sides is quite copious. Secondaries at the apices which originate from the midrib end together. The smallest areoles are present at the apex and largest in the middle region of the lamina. Vein endings are also of different shapes.

The epidermal cells show highly undulated walls. The mature stomata are diacytic. The trichomes are short with broad bases and tapering tips. The lithocysts are frequent (Fig. 1 N; Table II).

Adhatoda vasica of the subtribe Eujusticeae shows only one strand in the petiole which later organizes the midrib of the lamina. At the junction of the petiole and lamina it gives out very fine veins on either side which run at the margins and let out fine branches. The midrib gives rise to secondaries, alternately on the two sides. Some of the secondaries which start from the middle and uppei middle portion of the lamina reach the apex and ultimately open at the apex alongwith the tracheids of the midrib.

The areoles in the middle region are larger than those at the apex while the vein endings per areole are more numerous in the basal region (Table I). The characteristic feature of the areole of the apex is that the trached are ornamented (Fig. 2 A). The vein endings have broad ends since several tracheids join them at the apex (Fig. 2 B).

The walls of epidermal cells are sinuous and arched and the stomata are of diacytic type. The trichomes are multicellular and uniseriate, and the lithocysts are round to oval (Fig. 2 C, D).

Three species of Justiceae have been taken into account. In *Justicia betonica* one strand enters the petiole, right at the junction of the lamina and petiole and starts giving out secondaries at an angle of nearly 45°. The tertiaries come out at right angles to the secondaries. The tracheids show marked undulations. Plexuses are produced

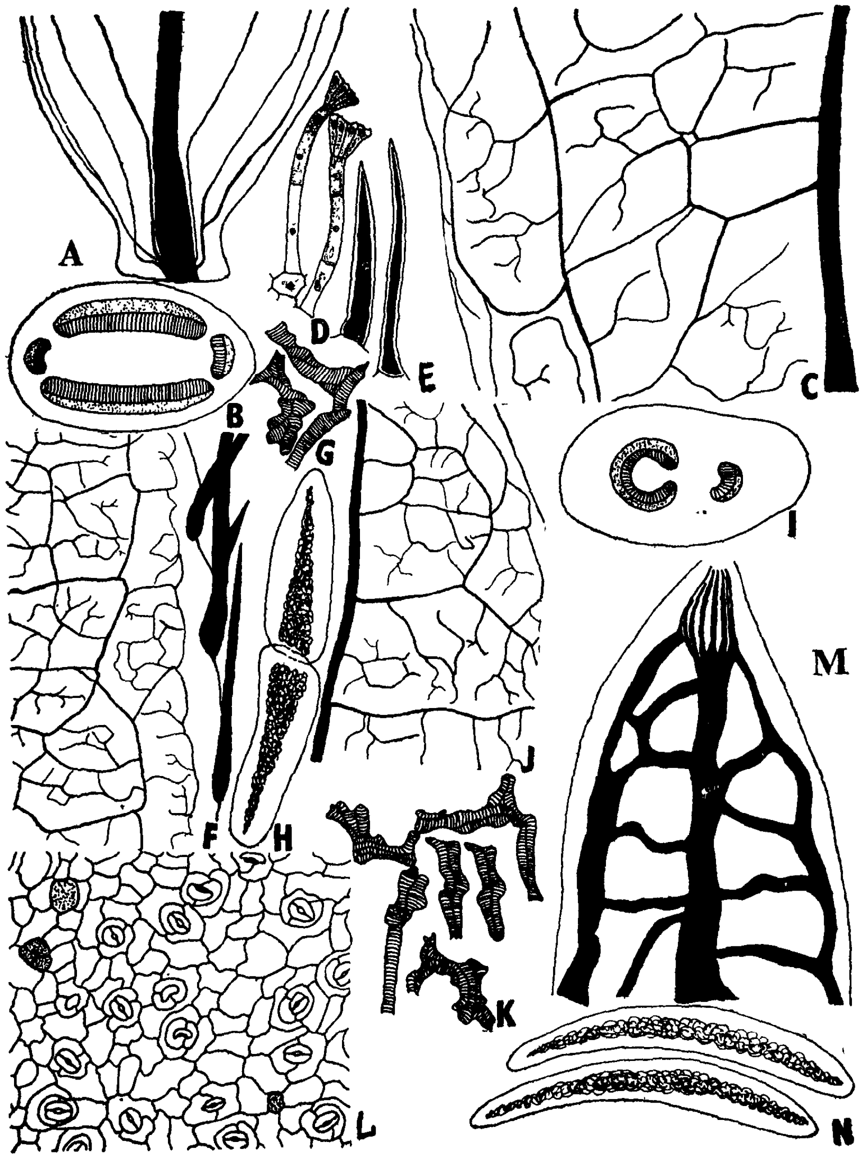

Fig. 1A-N : Vasculature and epidermal features of leaves of Andrographideae, Barlerieae and Asystasieae. A-E. Andrographideae-*Andrographis echioides*. A. Vascularization at the basal region. × 13. B. T. S. node. × 27. C. A small region of the leaf margin enlarged to bring out the areole organization. × 67. D & E. Multicellular, glandular and unicellular trichomes. ×267. F-L. Barlerieae-*Barleria prionitis* and *Crossandra infundibuliformis*. F-H. *Barleria prionitis*. F. Small portion of the leaf exhibiting marginal venation and trichomes. × 60. G. Vein tips. ×120. H. Cystolith. × 120. I-L. *Crossandra infundibuliformis*. I. T. S. node. × 13. J. Leaf venation; note the numerous vein endings and vein tips per areole. × 40. K. Vein tips. × 120. L. Portion of the lower epidermis with diamesogenous stomata and some abnormalities in the latter. × 75. M. & N. Asystasieae-*Eranthemum nervosum*. M. Apical region of the leaf magnified to show formation of compartments. × 67. N. Cystoliths. × 120.

on the margins. Some of the secondaries, which are given out in the apical region, form large compartments before reaching the apex. The opening of the lateral tracheids is in the same fashion as that of the midrib (Fig. 2 F).

The areoles are larger at the basal region. No direct correlation could be established between the number of vein endings per areole and the size of the areole (Table I). The vein endings are usually broad at the base and tapering at the apices (Fig. 2 G).

The cell wall of the epidermis is arched to sinuous; stomata being of the diacytic type. The trichomes are multicellular, uniseriate with tapering apices (Fig. 2 J). Lithocysts are very few, oval and very small (Table II).

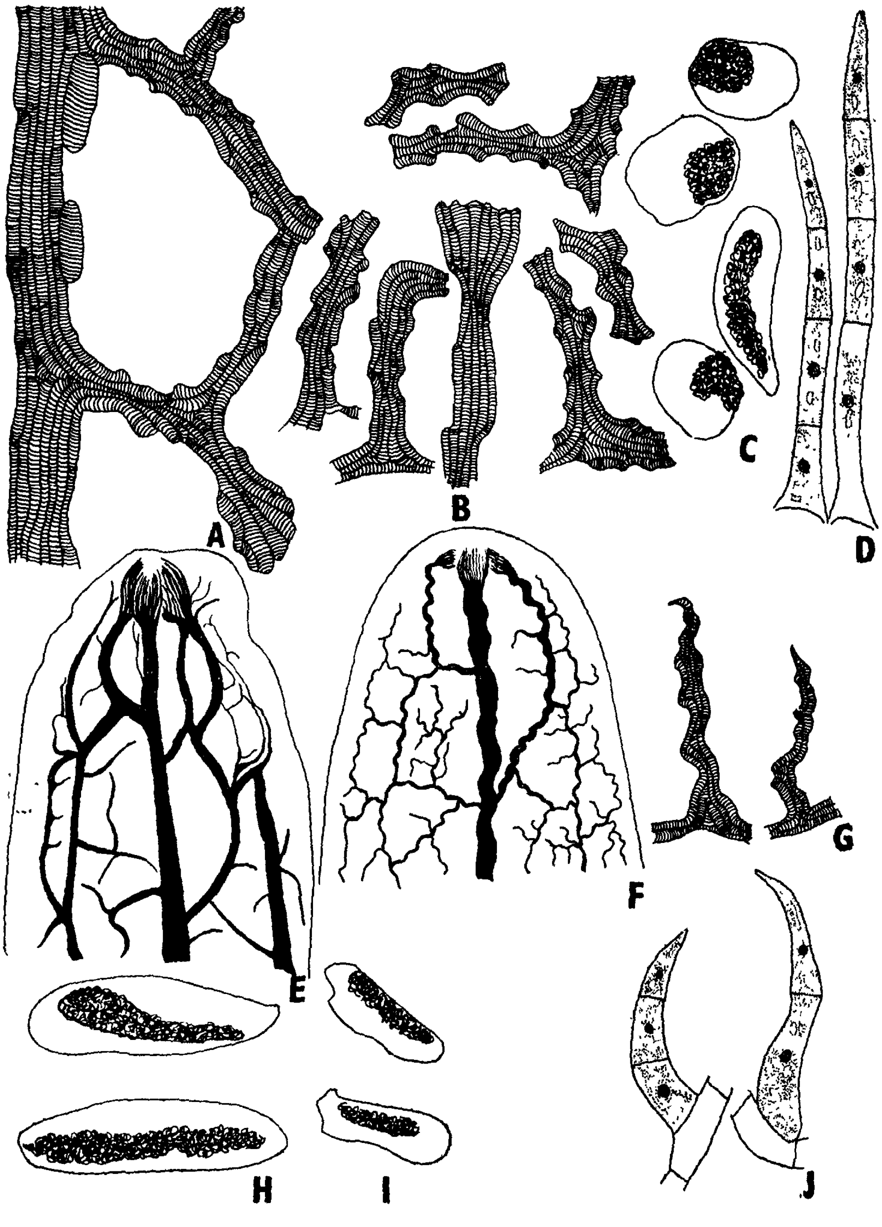

Fig. 2A-J : Foliar venation and epidermal features in Eujusticeae. A-D. *Adhatoda vasica*. A. Single areole enlarged to represent tracheidal accumulation. × 267. B. A few vein tips enlarged. × 267. C. Cystoliths. × 533. D. Two multicellular, uniseriate trichomes with tapering apices. × 533. E-J. *Justicia gendarussa*, *J. betonica* and *J. simplex*, respectively. E & F. Apical regions of *J. gendarussa* and *J. betonica* magnified. × 40. G. Vein endings of *J. betonica*. × 127. H & I. Cystoliths of *J. gendarussa* and *J. simplex*. × 267. J. Multicellular, uniseriate trichomes in *J. betonica*. × 267.

In *Justicia gendarussa*, a single strand enters the base of the petiole. It gives rise to secondaries at an angle of nearly 45°. The tertiaries are given out nearly at right angle to the secondaries. The secondaries form large compartments (Fig. 2 E). The plexuses are fewer as compared to those in *J. betonica.*

The areoles are smaller in the apical region but the number of vein endings per areole is the largest in this part whereas these are practically absent in the basal portion.

The epidermal cell walls are arched to sinuous being similar to those of *J. betonica.* The stomata are diacytic. The trichomes are of two types: multicellular, uniseriate with glandular head and multicellular, uniseriate with pointed apices. The lithocysts are oval to oblong (Fig. 2 D).

In *J. simplex* a single strand enters the base of the petiole which later gives out a fine vein on the left; soon after a second one is given out at the right side. Both of these enter the lamina and travel throughout the margins. The midrib gives rise to secondaries that give off tertiaries. The tracheids show undulations. The terminal ending of the tracheids is almost as that of *J. betonica.*

The areoles are of the same size at the base and apex while in the middle region they are smaller. The vein endings per areole are maximum at the apex.

The epidermal cell walls are arched to sinuous. Mature stomata are diacytic (Table II). According to the data collected, the frequency of the stomata is minimum in *J. simplex* and maximum in *J. betonica.* The lithocysts are quite long (*see* Fig. 2 I).

Peristrophe bicalyculata has a thick strand entering the petiole. It gives out fine, lateral branches. Before the junction of the lamina and petiole, it gives two fine secondaries on both sides to form big arches. They converge near the midrib. The midrib gives out a few additional secondaries. These also travel almost to the full length of the lamina. Some of them join the midrib again at the apex. The plexuses are frequently seen at the margins. The tracheids have spiral wall thickenings (Fig. 3 F).

The areole size is maximum at the apex and minimum at the base (Table I). The vein endings have several vein tips. These bear no direct correlation with the size of the areoles; vein endings usually comprise a single tracheid only.

The epidermal cell walls are sinuous. The stomata are of the diacytic type. The trichomes are multicellular, uniseriate with broad base and pointed apices. The lithocysts are elongated and the longest of the types studies so far (Fig. 3 C-E).

DISCUSSION AND CONCLUSIONS

Survey of the literature on the leaf anatomy has indicated that the features which are useful in taxonomic considerations include nodal organization, venation pattern, structure of the epidermis (stomata, trichomes, lithocysts), various types of crystals and laticifers. Further, these have been employed either singly or in conjunction with the characters drawn from disciplines other than morphology and anatomy.

Sehgal & Paliwal (1974) have divided the genus *Euphorbia* on the basis of venation pattern and grouped the various species into uni-, bi-, tri-veined, and special categories. Apart from this Kapoor *et al.* (1969) have classified the leaf characters into macro (all morphological characters) and micro (all epidermal characters), based on their studies of apocynaceous members. In the present investigation of tribe Justiceae, grouping has not been possible on the basis of veins entering the petiole. Regarding the venation pattern a very conspicuous single strand is seen in the petiole but in *Andrographis echioides* it gives rise to 6 traces. On the other hand, in *B. prionitis* three veins are seen, as the strand enters the petiole and in *Crossandra infundibuliformis* no branching of the main strand takes place. All the forms studied show single-strand condition and presence of fine branches in the petiole is not a regular feature.

Due to variability in the size of areole and number of vein endings, no direct re-

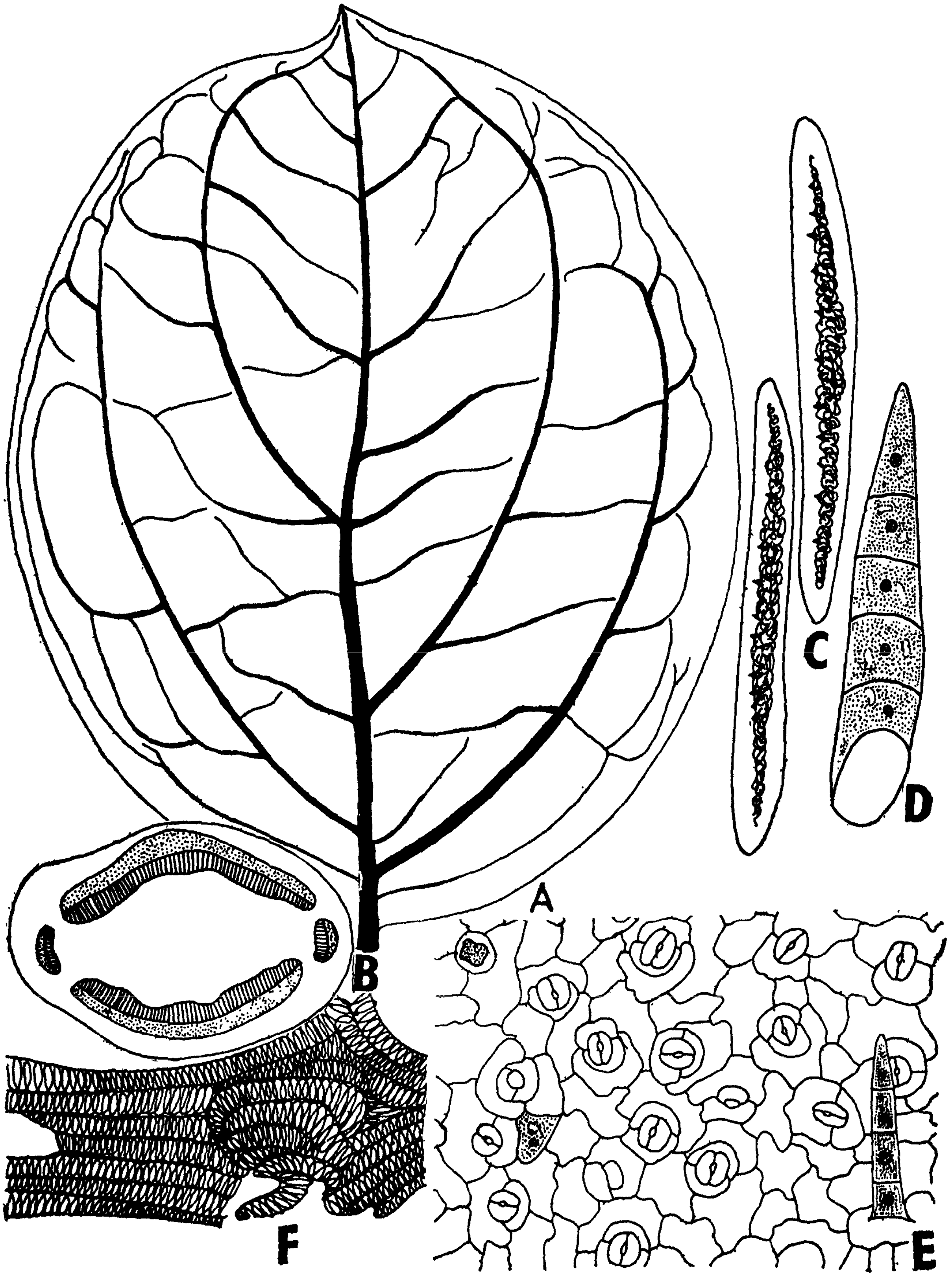

Fig. 3A-F : Foliar venation and epidermal features in Eujusticeae (contd.) A-F. *Peristrophe bicalyculata*. A. Gross leaf venation. ×2. B. T. S. node. ×20. C. Cystoliths. ×180. D. Single trichome. ×150. E. Portion of the lower epidermis. × 113. F. Plexus formed by the vein commissures at the margins. × 400.

TABLE I

MORPHOLOGICAL CHARACTERS OF THE LEAVES OF TRIBE JUSTICEAE (FAMILY ACANTHACEAE)

Species	Phyllotaxy	Size sq. mm	Shape	Margin	Apex	Node	Areole size sq. mm	No. of vein endings/per areole
Andrographis echioides	opposite, decussate	90	lanceolate	entire	acute	unilacunar	*A. 024 *M. 102 *B. 329	0.6 0.6 1.0
Barleria prionitis	do	67	do	do	do	do	A. 017 M. 016 B. 017	0.2 1.0 1.2
Crossandra infundibuliformis	do	411	ovate	do	do	do	A. 039 M. 027 B. 043	0.2 0.2 1.0
Eranthemum nervosum	do	239	lanceolate	do	do	do	A. 014 M. 052 B. 024	0.8 0.8 0.6
Adhatoda vasica	do	586	do	do	do	do	A. 047 M. 074 B. 062	1.0 1.0 1.6
Justicia betonica	do	89	cordate	do	do	do	A. 023 M. 010 B. 067	1.2 1.6 1.6
J. gendarussa	do	151	lanceolate	do	do	do	A. 042 M. 069 B. 069	0.8 0.2 0.0
J. simplex	do	96	do	do	do	do	A. 12 M. 07 B. 12	2.2 1.0 1.0
Peristrophe bicalyculata	do	955	do	do	do	do	A. 064 M. 049 B. 023	0.8 0.6 0.8

*A-Apex ; B-Base ; M-Middle.

TABLE II

LEAF EPIDERMAL CHARACTERS OF THE TRIBE JUSTICEAE (FAMILY ACANTHACEAE)

Species	Topography of the cell wall	No. of epi. cells (sq. mm)	Frequency of stomata (per sq. mm)	Stomatal Index	Trichome shape	Cystolith shape
Andrographis echioides	feebly undulated	666	306	20.5	uniseriate multicellular with glandular apex, unicellular nonglandular with pointed apex	elongated
Barleria prionitis	straight to arched	1200	90	50.0	unicellular with tapering apex	bi-celled with tapering apex
Crossandra infundibuliformis	arched to sinuous	866	426	33.0	uniseriate, multicellular with pointed apex	elongated but scanty

Contd.

Species	Topography of the cell wall	No. of epi. cells (sq. mm)	Frequency of stomata (per sq. mm)	Stomatal Index	Trichome shape	Cystolith shape
Erathemum nervosum	highly undulated	440	293	60.0	non-glandular, two-celled with broad base and pointed apex	sausage-shaped
Adhatoda vasica	sinuous to arched	1120	253	18.0	uniseriate, multicellular	oval to round
Justicia betonica	arched to sinuous	76	360	64	uniseriate, multicellular, pointed apex	oval, small in size
J. gendarussa	do	666	200	23	short, stalked with glandular head and uniseriate, multicellular, pointed apex	oval to oblong
J. simplex	arched to sinuous	800	163	17.8	uniseriate, multicellular, pointed apex	elongated
Peristrophe bicalyculata	sinuous	560	253	30.6	uniseriate, pointed apex and broad base	elongated

lationship could be established between the two. In this contest, we support Richardson's 1970 suggestion that venation is particularly susceptible to the ecological conditions. Levin (1929) attached great systematic importance to vein islets areas. According to him, the vein islet number varies within narrow limits and is thus sufficiently constant in different species. Later, Hall & Melville (1951, 1954) opined that the number of veinlet terminations, either alone or in conjunction with other histological characters is of taxonomic value particularly in genera with only a small number of species. However, Sehgal & Paliwal (1974) could not establish any direct correlation between the size of areole and number of vein endings in the areole in family Euphorbiaceae. Present investigations also lend no support to Hall & Melville's viewpoint.

According to Metcalfe & Chalk (1950), the stomata of the family Acanthaceae are of caryophyllaceous type. From the present study also it is clear that the stomata are uniformly diacytic (caryophyllaceous; Ahmad, 1974).

The types of trichomes recorded here have also been reported by Inamdar (1970) and Ahmad (1974). The lithocysts are present in all the species studied. They exhibit little variation in shape and size but in *B. prionitis* these are bi-celled, a type which has not been observed in other forms.

Following characters are common to all the species studied opposite, decussate phyllotaxy of leaves, unilacunar node, a single prominent strand, presence of two types of trichomes, lithocysts and caryophyllaceous stomata. Thus these appear to form a good basis, worthy of consideration in any taxonomic grouping.

On the other hand, the venation pattern, areole size and number of vein endings are features which cannot be taken into account since variation exists not only in the different species but in the same lamina also. The present observations tend to support the views of Sehgal & Paliwal (1974).

It would not be out of place to comment on the delimitation of Acanthaceae as suggested by Bremekamp (1965). His remark that subtribe Andrographoideae should be separated from Justiceae on the basis of embryological characters stands negated since the venation and epidermal characters present in *A. echioides* are identical to those occurring in other members of the tribe Justiceae.

Datta & Maiti (1969) have also attempted to discuss the taxonomic interpretations on the basis of floral anatomy of the tribe Justiceae (Acanthaceae). According to their observations, *Barleria* has a tendency of early branching. In the present investigations also early venation has been observed in this taxon-hence it has been described as triveined. Our observations provide support to their conclusions. Further, on the basis of the cytology and floral anatomy also Datta & Maiti (1971) have suggested a separation of *Barleria* from Justiceae. The totality of characters thus indicates that *Barleria* stands apart from other taxa of the Justiceae.

However, several lacunae exist in our knowledge, particularly in respect of the extent to which the climatic factors influence the various features associated with the veins and of importance of venation features.

Work on these and related lines is in progress in this laboratory and it is hoped that a complete study of other tribes of the family will help to provide a more complete picture for taxonomic interpretations.

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